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## ABSTRACT

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Metabolic studies of "hepatorenal syndrome" indicate that limitation of urine flow rate in cirrhotics correlates with reduced GFR and resultant decreased urinary solute excretion. The decreased GFR is not due to co-existing renal disease or decrease in cardiac output. Preliminary studies on renal blood flow, PAH extraction and tubular functions suggest that a primary renal hemodynamic abnormality may be involved. This possibility is being considered. An evaluation of the clinical and pathological characteristics of renal failure in 117 cirrhotics is also in progress. Anemia is a common accompaniment of hepatic coma, but these patients often are not hyperkalemic. Laboratory tests permit differentiation of "hepatorenal syndrome" from other causes of renal insufficiency. Decreases in GFR may occur suddenly without relation to known causes, and are often preceded by an episode of hepatic coma. Treatment of the coma with neomycin, laxatives, enemas and dietary protein restriction are not causally implicated.

The relation of potassium deficiency to metabolism of ammonia and amino acids is the subject of the following current research. A dog preparation involving induction of potassium deficiency by dialysis is now being used to extend our clinical studies on the relation of potassium and ammonium metabolism in kidney (J. Clin. Invest. 42: 696, 1963). The relation of potassium deficiency to alterations in amino acid composition of tissues is being studied in groups of rats. Significant increases of threonine, serine, glycine, alanine, lysine and arginine occur in muscle of electrolyte depleted animals. The significance and mechanisms of this change in amino acid pattern are being investigated.

A study on the effects of urea and ammonium chloride infusions on gastric ammonium concentration and on secretory responses to histalog has been completed. The results indicate that blood ammonium is a significant source of gastric ammonium, possibly more so than blood urea. Results were similar in neomycin treated and in non-antibiotic treated patients. Gastric secretory responses were not altered by elevations of blood or gastric juice urea or ammonium levels, or by pre-treatment with neomycin. Ammonium had no evident neutralizing action on normal human gastric juice.

Copies of this report are filed with the Armed Services Technical Information Agency, Arlington Hall Station, Arlington 12, Virginia, and may be obtained from that agency by qualified investigators working under Government Contract.

**PROGRESS REPORT - TO THE SURGEON GENERAL**

**DEPARTMENT OF THE ARMY**

**Contract No. DA-49-007-MD-749**

**May 1, 1962 - April 30, 1963**

**Metabolism of Protein, Amino Acids and  
Ammonia in Patients with Liver Disease**

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**Supported by:**

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Studies supported by this contract are presented under three major headings: 1) Metabolism of Ammonium, 2) Renal Failure Associated with Hepatic Disease, 3) Metabolic Aspects of Potassium Deficiency. For each research endeavor recent progress is cited, work to date is summarized briefly, then current status is indicated.

### I. Metabolism of Ammonium

The origin, source and significance of ammonium in gastric juice has been investigated in these laboratories during the past two years. These studies were designed to determine the influence of urea and ammonium chloride infusions on gastric ammonium concentration and on gastric secretory responses to histalog. The responses of seven patients given ammonium chloride infusions and eight patients given urea infusions had been determined previously. During the past year four additional patients were studied with ammonium chloride and six with urea given intravenously after pre-treatment with the nonabsorbable antibiotic, neomycin. The neomycin was used to evaluate the role of gastrointestinal bacteria on ammonium content of gastric juice and permit an evaluation of whether or not bacterial urease was significantly involved. Comparison of data obtained during initial control periods from subjects pre-treated with neomycin with those obtained from subjects not given antibiotic demonstrates the following: The gastric juice values for pH, volume of secretion, ammonium and urea nitrogen levels did not differ significantly. Comparisons of each of these values for the neomycin group with those obtained for the nonantibiotic group yielded  $P < 0.1$ . However, blood ammonium nitrogen concentration was decreased and blood urea nitrogen level increased significantly in the neomycin-treated group as compared to the group not given antibiotics ( $P = < 0.05$ ). These findings may reflect the effect of neomycin on urease containing organisms in the lower gastrointestinal tract. The gastric secretory responses to betazole hydrochloride (histalog) were not influenced by neomycin pre-treatment. The increases in blood and gastric juice ammonium nitrogen concentrations that resulted from ammonium chloride given intravenously were similar in the patients pre-treated with neomycin and in those not given antibiotic. Similarly, the results of urea infusions in the patients who did not receive antibiotic were similar to the findings in those who were treated with neomycin.

As an additional control experiments, the result of ammonium chloride infusions were studied in two patients with pernicious anemia. These patients had histalog-fast gastric anacidity. They were infused with ammonium chloride according to a protocol similar to that used for the other subjects. The infusion produced sustained elevations of arterial blood ammonium nitrogen levels, but urea nitrogen levels were unchanged. Gastric ammonium nitrogen levels increased. These patients demonstrated the expected poor gastric secretory responses to histalog. The increases in gastric juice ammonium nitrogen concentration and content produced by the ammonium chloride infusions were unchanged following histalog, an effect different from the changes noted in the other subjects studied who had normal gastric secretory mechanisms. This latter study indicates that the influence of histalog on increasing gastric juice ammonium nitrogen concentration is related to the gastric secretory response and not to histalog per se.

The studies to date may be summarized as follows: The effect of ammonium chloride and urea infusions on gastric juice ammonium and urea levels were compared. Ammonium chloride infusions raised blood and gastric juice ammonium levels without affecting blood or gastric juice urea levels. The transfer of ammonia from blood to gastric juice was accelerated by stimulation of gastric secretory activity by betazole hydrochloride. The hypothesis is advanced that gastric juice ammonium is derived in part from blood ammonium and that ammonia diffuses into and is trapped in the acid gastric juice. Ammonium chloride infusions produced larger increases in gastric juice ammonium than did urea infusions even though the nitrogen content of the infused urea was 15 times greater. Urea infusions produced increases in gastric juice ammonium without changing blood ammonium levels. Betazole hydrochloride did not accelerate the rise in gastric juice ammonium with urea infusion. Pre-treatment with neomycin did not alter the action of either infusion in producing gastric juice ammonium. It was concluded that gastric juice ammonium is also derived from blood urea, hydrolyzed by a tissue rather than a bacterial urease. The relative contribution of blood urea and ammonium to gastric ammonium content in the normal is unknown. The gastric secretory response to betazole hydrochloride was not significantly inhibited by either elevation of blood or gastric juice urea or of blood gastric juice ammonium, or by pre-treatment with neomycin. Gastric juice ammonium had no evident neutralizing action on normal human gastric juice.

A completed manuscript describing these findings has been submitted for publication.

## **II. Renal Failure Associated with Liver Disease**

Two types of investigations are involved in the studies on the renal failure associated with liver disease. The first concerns detailed metabolic studies of the factors influencing maximal urine flow rates in patients with cirrhosis. Preliminary findings were given in last year's report of progress. During the past year a second study dealing with the clinical and pathological characteristics of this type of renal failure was initiated.

### **A. Maximal Urine Flow**

The studies of factors limiting flow rate in cirrhotics were accomplished under conditions of controlled sodium, protein and fluid intakes in the Metabolic Unit. Thirty-four patients were observed, including eight with preterminal oliguria and azotemia. As indicated previously, a correlation between ability to excrete a water load and ultimate prognosis was confirmed. Inability to increase urine flow rate above 3 cc per minute is a grave prognostic sign. Patients with low urine flow rates had reduced osmolar and free water clearances and decreased glomerular filtration rates (GFR). A final evaluation of these data justifies the following comments: There were significant correlations between rates of urine flow and solute excretion, urine flow and GFR, and GFR and solute excretion. Application of the statistical method of partial correlation showed that reduced GFR by reducing urinary solute excretion accounted for oliguria in most cirrhotics. Data from

a separate study on the effects of solute loading supported this conclusion. Patients with low GFR had reduced  $C_{PAH}$  and  $Tm_{PAH}$  without increases in filtration fractions. Their urine was usually hypertonic to serum and contained less than 10 mEq of sodium per liter. Renal lesions were not detected by light microscopy in this group. This renal functional pattern suggested that reduced GFR and oliguria in cirrhotics are due to either afferent arteriolar constriction or shunting of blood away from the kidney. Since cardiac index is normal or increased in these patients, reduced cardiac output cannot be invoked to explain the decreased GFR. A demonstration by others of a reduced total renal blood flow by the nitrous oxide technique suggests that diversion of a significant portion of cardiac output away from the kidneys may occur. However, reduced renal blood flow occurring with peripheral arteriovenous shunting or abnormal pooling of blood is usually associated with increased afferent arteriolar resistance and increased filtration fractions. These parameters were measured in three patients in our series and were normal or reduced. Increased afferent arteriolar resistance cannot be excluded as a mechanism for diverting blood from the kidney.  $PAH$  extractions were studied in three of the patients and these were reduced. This suggests an abnormal distribution of blood within the kidney as a cause for the decreased GFR. None of these had sufficient histologic evidence of chronic renal disease to explain the reduced  $PAH$  extractions. Tubular function was also grossly intact as estimated by ability to clear the urine of sodium and by high creatinine U/P ratios. The possibility that in the renal failure associated with liver disease blood is being shunted within the kidney but away from functioning renal mass is being considered.

Work in this particular area has been temporarily disrupted because of Dr. Leroy Shear's call to active duty at WRAIR. We hope to resume this type investigation in July when new Research Fellows will be joining us. A manuscript on the data obtained to date has been submitted for publication.

### B. Clinical and Pathological Characteristics of Renal Failure in Cirrhosis.

This study involved material obtained from two sources. Clinical records of 117 patients who died in Metropolitan General Hospital between January, 1958 and October, 1962 with clinical diagnoses of hepatic cirrhosis were reviewed. Data from this retrospective analysis were supplemented by planned metabolic studies in 15 patients with diffuse hepatic disease complicated by azotemia. Eight of these patients were maintained in the Metabolic Unit where quantitative diet control and collections could be insured, within the limits of providing adequate treatment. These patients were carefully observed from time of admission to time of death. Seventy-three patients dying with cirrhosis demonstrated hepatic coma. Eighty-four per cent of the patients with hepatic coma had elevated BUN. Severe azotemia ( $BUN > 50 \text{ mg\%}$ ) was noted in 32 patients with cirrhosis without co-existing cardiac or renal disease. All but one of these had hepatic coma. Serum sodium concentration was measured shortly before death in 73 patients with cirrhosis and azotemia in whom the presence or absence of hepatic coma could be established. Most died with serum sodium concentrations which were normal or only slightly reduced. Evaluation of the 14 patients with severe

hyponatremia (sodium less than 125 mEq/liter) indicated that significant hypotonicity usually was due to overhydration. By contrast to patients without cirrhosis dying from renal failure, the patients with cirrhosis and azotemia in the present report generally were hypo or normokalemic. When hyperkalemia developed it was usually mild and in 3 of 8 patients was clearly related to excessive potassium administration. Thus hyperkalemia usually is not a serious problem in patients with cirrhosis and renal failure unless potassium is administered.

The prospective study of 15 patients has provided useful information. This study shows that renal failure developing in patients with cirrhosis may be related to pre-existing renal disease, acute tubular necrosis (secondary to hypotension, blood loss, etc.,) or to renal failure that is not identified with any known cause and in which acute tubular necrosis as a pathogenetic explanation is most unlikely. Study of 5 patients in the last category demonstrate the following: GFR was reduced before azotemia was apparent in all 5 patients. Further reductions in GFR, often occurring suddenly, were followed by increases in BUN and serum creatinine concentrations. These were not associated with decreases in body weight or changes in hematocrit, hemoglobin, or serum electrolyte concentrations. After GFR was already decreased, creatinine U/P ratios were high, urinary sodium concentrations were low and osmolar U/P ratios exceeded 1. All of these functions tended to deteriorate terminally. Changes in tubular function were associated with preterminal hypotension in all 5 patients, but again GFR had clearly decreased prior to the development of the change in blood pressure. Thus the post mortem finding of tubular lesions has little bearing upon the pathogenesis of the renal failure if tubular disease is not borne out by preterminal functional tests. The data from the 15 patients who developed renal failure while under observations permit certain speculations. Some patients have tubular necrosis, others demonstrate extrarenal abnormalities which may contribute to renal failure. These abnormalities in general are not extreme enough to produce frank renal failure in patients with normal kidneys. Patients with cirrhosis, however, frequently demonstrate reduced GFR's and renal plasma flows before renal failure develops. They therefore may respond like patients with chronic renal disease who are very susceptible to small variations in cardiac output, hydration, perfusion pressure, etc. Thus patients with cirrhosis may have an increased susceptibility to the development of renal failure. Five patients died with acute renal failure even though the usually recognized causes were excluded. Light microscopy did not reveal evidence of chronic renal disease or glomerulitis. These patients also did not have proteinuria. Interestingly, histologic evidence of some tubular damage was found at post mortem examination in most instances. Previous studies indicate that impaired renal function in patients with liver disease may be due to a primary vascular abnormality characterized either by diversion of blood away from the kidneys or by intrarenal shunting. The findings in this prospective study of suddenly decreasing GFR, tend to support these impressions, and point to the need for additional study of the renal hemodynamics in patients with cirrhosis. A high degree of association between the presence of hepatic coma and the development of renal failure has been confirmed. A high incidence of renal failure occurred in patients dying with hepatic coma. Moreover, in 4 of the



5 patients reductions in GFR occurred in direct relation to episodes of hepatic coma. Thus interpretation of the relationship between hepatic coma and renal failure must remain speculative. The 5 patients cited developed coma prior to the development of renal failure. Therefore, precipitation of coma by increased concentrations of urea, etc. in the blood may not be the only explanation for this association. There is no indication from these studies that treatment of coma with orally administered neomycin, laxatives, enemas and dietary protein restriction could be implicated as being causative.

The data obtained from this clinical and pathological study are being evaluated further and a manuscript is in preparation.

### III. Potassium Metabolism

#### A. Potassium-Ammonia Relation in Kidney

The results of clinical studies (to be published in May, 1963, Journal of Clinical Investigation) demonstrate that acute potassium depletion influences metabolism of ammonium by kidney. This study demonstrates that with potassium deficiency either renal production of ammonium or availability of this ion to tubular fluid and renal venous outflow is increased. Current studies are directed to determining the exact source of the ammonium and the role of potassium in its regulations. Two possibilities are being considered: 1) That renal ammonium production is increased as a result of increased glutaminase activity, and 2) that renal ammonium production is unaltered by potassium deficiency, but availability of ammonium produced in tubular cells is affected by the presence or absence of potassium through some influence the latter has on permeability of tubular cell membranes. Recently laboratory investigations utilizing a dog preparation have been initiated to elaborate these. The preparation involves the induction of potassium deficiency by dialysis on an artificial kidney, continuous monitoring of blood pressure and renal blood flow, and serial determinations of blood pH, potassium and other electrolytes, serial collections of urine for similar determinations and frequent sampling of blood for ammonium levels from artery, peripheral vein and renal vein. Several preliminary experiments have been done. Control observations indicate that ammonia metabolism by kidney is not altered by severe respiratory acidosis, or by dialysis without inducing potassium deficiency, and that induction of potassium deficiency by dialysis will induce changes in ammonium metabolism similar to those described for patients. Accordingly, this should provide a good experimental model for further work. If a relation between potassium and ammonium metabolism by kidney can be established with this preparation, it is planned also to obtain kidney tissue for various appropriate in vitro studies.

#### B. Effects of Single Amino Acids on Metabolism of Potassium Hydrogen Ion.

Past reports of progress have outlined metabolic studies in patients with and without liver disease that indicate an effect of basic amino acids (arginine, lysine) on the metabolism of potassium and hydrogen ion. More recently these observations have been extended utilizing rats as the experimental animals. The effects of dietary sodium and potassium restriction, and of L-lysine loading on blood, urine and tissue electrolytes were determined. Muscle analyses for amino acid changes were also done. The preliminary results are as follows: Animals fed electrolyte free diets as

compared to electrolyte fed animals showed increase in urine pH, decreases in urine sodium and potassium, and increases in ammonium excretion. Net acid excretion was twice the acid excretion for the control group. Accordingly, blood pH increased and bicarbonate values increased in the electrolyte-deficient animals. Plasma sodium, potassium, and chloride concentrations also decreased. When the electrolyte fed animals were given L-lysine in addition to the diet, urine pH and electrolytes did not change except for potassium excretion which increased significantly, a finding similar to that noted in the human subjects previously studied. Blood electrolytes were not changed. When the animals depleted of sodium and potassium were loaded with L-lysine there were no significant changes in the blood or urine electrolytes. Initial complete analyses of muscle obtained from animals given diets containing sodium and potassium and L-lysine loaded indicate decreases in intramuscular pH, sodium and potassium contents. The amino acid patterns of muscle using the method of Stain and Moore were completed for a few of the animals. Significant increases in intramuscular concentrations of threonine, serine, glycine, alanine, lysine and arginine occurred in muscle of the electrolyte depleted rats as compared to muscle of control animals. Fifteen other amino acids studied were unchanged as a result of electrolyte depletion. When the control animals were compared with animals given lysine, the latter demonstrated increased muscle concentrations of glycine, alanine, lysine and arginine. The potassium and sodium depleted rats were given lysine and compared with potassium and sodium depleted controls. There were significant decreases in asparagine and glycine but no apparent changes in intramuscular lysine or arginine concentrations.

Another experiment involving animals has been completed utilizing a similar pair feeding protocol, but on this occasion involving sodium and potassium depletion in separate groups of animals. Other groups of rats on the various basic diets employed have been given loads of glycine and of glutamic acid. As of this writing, the tissue analyses for electrolytes and for amino acids which are the most critical parameters have not been adequately completed and accordingly results are not presented. The findings of changes in muscle amino acid patterns with electrolyte depletion and with amino acid feeding encourage additional studies of this type, tedious as they are.

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